REMARKS

Claims 1 through 16 remain in the application. Claim 17 is newly added, and no new matter is contained in these amendments.

Applicants submit the present amendments and remarks, and respectfully request reconsideration and allowance of the remaining claims.

Rejection Under 37 C.F.R. 1.75(c)

The Examiner rejected claims 2 and 4-16 under 37 C.F.R. 1.75(c), for being in improper form because a multiple dependent claim cannot be dependent on another multiple dependent claim. Applicants have amended claims 4-6, 9-12 and 14 in this regard, and the rejection should be removed.

The Examiner objected to claim 13 because it appeared to be a Markush claim in improper format. Applicants have amended claim 13 to address this objection.

Rejection Under 35 U.S.C. § 112

The Examiner rejected claims 5, 9 and 15-16 under 35 U.S.C. § 112 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants have amended the claims to remove the term "briefly" in claim 5, "in particular" in claim 9, and to clarify that the processes claimed in claims 15 and 16, and the rejection should be removed.

Rejection Under 35 U.S.C. § 102(e)

The Examiner rejected claims 1-6 and 9-16 under 35 U.S.C. §103(a) as obvious over Takeda et al. or Smithey et al. in view of Gaffney, Dutton et al., McDowell et al., Kobayashi et al. and Lehninger et al.

The present application is directed to a process for the production of covalently cross-linked bacteriorhodopsin, wherein bacteriorhodopsin is used in membrane-bound form as a substrate of a transglutaminase. A primary advantage of the claimed method is that it avoids the use of low-molecular linker molecules as conventionally used for covalent coupling of purple membranes. Since the coupling sites of the bacteriorhodopsin molecules in purple membranes (which are crystals) are poorly accessible, it is very surprising that according to the present invention, almost 100% coupling efficiency is achieved, as illustrated, for example, in Fig. 3 of the present application. The bands of the starting molecules (Fig. 3A) disappear completely in the course of the reaction and the product is quantitatively formed (Fig. 3F). Moreover, cross-linking according to the invention is characterized by a simple procedure compared with the complicated processes of the prior art. Simple mixture of bacteriorhodopsin and transglutaminase, and optionally further substances, leads to the formation of the desired products. Moreover, staples of several layers of bacteriorhodopsin purple membrane in direct order can be produced according to the present invention, which generate high voltages when exposed to light and, for example, can be used as switches or control elements.

Takeda et al. is directed to a process for preparing a protein-orientated membrane. According to Takeda et al., cross-linking of bacteriorhodopsin is carried out using glutaraldehyde and similar low-molecular linkers (cf., for examples, claims 7 to 9). Prior to cross-linking, an orientation of proteins via electrophoresis or antigen-antibody reaction must be effected (cf., for example, column 2, line 67 to column 3, line 3). Consequently, Takeda et al. describe a very complicated method using low-molecular linker molecules which are avoided according to the present invention (see for example, present application, page 2, second paragraph).

Smithey et al. disclose bacteriorhodopsin preparations having increased information storage times, wherein the bacteriorhodopsin materials are crosslinked using monomeric crosslinkers such as formaldehyde, dialdehydes, diamines and the like (see column 4, lines 48 to 51). However, the cross-linking as performed according to Smithey et al. represents the conventional way of cross-linking bacteriorhodopsin using low-molecular linker molecules which are avoided according to the present invention.

Gaffney is an overview of the chemical and biochemical cross-linking of various membrane components. Thereby, Gaffney first described in detail the use of low-molecular cross-linkers (pp. 294-299) as well as cross-linking via photoactivatable cross-linkers (pp. 299-304). Page 304, bottom of left column to right column, third paragraph, deals with biochemical transglutaminase cross-linking. Gaffney does not give any precise teaching regarding which molecules can be linked by transglutaminase. It is only stated that cross-linking of cytoskeleton and membrane proteins is observed when adding calcium. The cross-linking of

bacteriorhodopsin or even bacteriorhodopsin in membrane-bound form is neither described nor suggested. It is significant to note that bacteriorhodopsin in the form of purple membranes are protein-lipid crystal structures, while erythrocyte membranes as used in Gaffney (see page 304, left column, last paragraph and right column, last sentence of first paragraph) are flexible, fluid structures having different properties. In particular, binding sites are considerably more accessible in flexible membranes than in crystal structures. Thus, results obtained for erythrocyte membranes cannot be simply applied to bacteriorhodopsin in membrane-bound form.

On page 311, left column, Gaffney explicitly describes cross-linking of bacteriorhodopsin, and in that case solely small molecules, namely photoactivatable cross-linkers, are used to effect cross-linking, as was common practice and considered necessary in the prior art before the present invention. Thus, Gaffney mentions both transglutaminase and bacteriorhodopsin, however, in a different context and not in combination. Without taking a hindsight approach, knowing the present application, the skilled person could not gather any hint from Gaffney that such combination would be possible. In fact, the lack of recognition by Gaffney of the advantages of the present invention of combining transglutaminase and bacteriorhodopsin indicates its nonobviousness.

Thus, by combining Takeda et al. or Smithey et al. with Gaffney, the skilled person could not arrive at the present invention, according to which cross-linking of bacteriorhodopsin in membrane-bound form is possible by means of transglutaminase, without requiring the use of low-molecular linker molecules. Rather, the cited documents lead away from the present

invention, because in either case linker molecules are taught to be necessarily used for cross-linking bacteriorhodopsin.

The further documents cited by the Examiner do not add anything to Takeda et al., Smithey et al. and/or Gaffney which would enable the skilled person to arrive at the present invention, as indicated below.

Dutton et al. disclose the cross-linking and labeling of membrane proteins by transglutaminase catalyzed reactions. In particular, Dutton et al. discuss cross-linking and labeling experiments using mouse erythrocyte membranes (see page 2568, first paragraph and "Materials and Methods" on the same page). Bacteriorhodopsin is not mentioned in Dutton et al. Moreover, as outlined above, bacteriorhodopsin in membrane-bound form substantially differs from erythrocyte membranes in that it represents a crystalline structure, whereas erythrocyte membranes are flexible, fluid structures having substantially different properties. No suggestion can be found in Dutton et al. that it would be possible to use transglutaminase for the cross-linking of membrane-bound bacteriorhodopsin. Rather, one skilled in the art searching for an improved method for specifically cross-linking bacteriorhodopsin in membrane-bound form would not take Dutton et al. into account at all and would not combine this document with any of the documents referring to bacteriorhodopsin.

McDowell et al. disclose the modification of rhodopsin by means of transglutaminase. In particular, transglutaminase is used to attach small amines to rhodopsin in order to investigate its topography and function. Consequently, McDowell et al. relate to the investigation of rhodopsin,

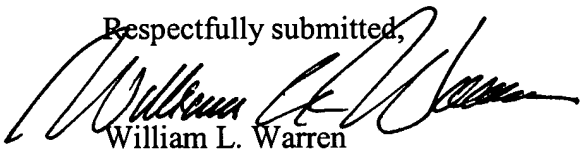
which represents a completely different technical field compared to the present invention referring to the cross-linking of bacteriorhodopsin. Further, the small molecules that are attached to rhodopsin using transglutaminase according to McDowell et al. are quite mobile due to their low molecular weight. Therefore, the molecules diffuse to the membrane and react there. Contrary to the attachment of small molecules as in McDowell et al., according to the method of the present invention, cross-links between membrane-bound bacteriorhodopsin molecules are achieved, which means that complete membrane patches are coupled together. Such membrane-bound proteins when used in water are not dissolved but form a dispersion. For example, a purple membrane typically contains 10,000 to 30,000 bacteriorhodopsins. This aggregate shows an extremely low Brownian motion. Since the Brownian motion will not bring the "second" membrane to the "first" membrane, as is the case when small molecules are attached, it is highly surprising that transglutaminase can be used to covalently cross-link bacteriorhodopsin without linker molecules as achieved according to the present invention. McDowell et al. do not even address the problem underlying the present invention and one skilled in the art cannot take any hint from McDowell et al. as to the use of transglutaminase for cross-linking bacteriorhodopsin in membrane-bound form as claimed in the present invention.

Kobayashi et al. disclose a process for producing a transglutaminase and Lehninger et al. refer to investigations with respect to the temperature dependency of enzyme catalyzed reactions. Both documents are irrelevant for the claimed subject matter of the present invention.

In summary, the subject matter of the present invention is neither disclosed nor rendered obvious by any of the cited documents or by any combination of the documents if the skilled person would have combined them at all. Rather, the combination of the documents as suggested by the Examiner represents an inadmissible hindsight approach knowing the subject matter of the present invention. Therefore, Applicants respectfully request withdrawal of the prior art rejections and allowance of the claims.

The Examiner is encouraged to call the undersigned attorney at 404-853-8081 if doing so will facilitate prosecution of the application. No fees are believed to be due at this time. However, the Commissioner is hereby authorized to charge any additional fees due or credit any overpayment to Deposit Account 19-5029.

Respectfully submitted,



William L. Warren
Registration No. 36,714

SUTHERLAND ASBILL & BRENNAN LLP
999 Peachtree Street, NE,
Atlanta, Georgia 30309-3996
Telephone: 404.853.8000
Atty Docket: 18744-0001